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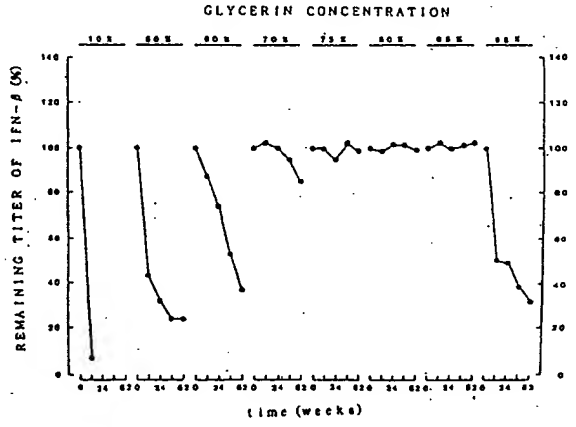
(54) **STABLE INTERFERON -g(b) COMPOSITION.**

(57) A stable interferon β composition is disclosed, which contains 65 to 90 wt % of polyol and a p-hydroxybenzoate. This composition can be stored for a long time at room temperature and, even when various additives are incorporated therein, IFN β will not be deactivated and the IFN β stabilizing effect will last for a long time.

FIG 1



EP 0 374 257 A1

DESCRIPTION

STABLE COMPOSITION OF INTERFERON-β

TECHNICAL FIELD

This invention relates to the stable composition of interferon-β (IFN-β) which is designed for topical or systemic administration, and especially relates to a stabilized composition of IFN-β which is designed for use as an ointment.

BACKGROUND ART

The method of the mass production of IFN-β, which inhibits virus reproduction, was developed using cell culture and DNA recombinant technology. IFN-β produced by the former method is already commercially available. Although highly refined IFN-β is in demand as a pharmaceutical agent, there is a problem in that the more refined it is, the less stable it becomes. The uses of polyol (Japanese Patent Application Laied-Open (Kokai) No. 92619/83 and so on), human serum albumin (International Publication No. W083/01198), organic acid buffer (Japanese Patent Application Laied-Open (Kokai) No. 92621/83), carboxymethyl cellulose (Japanese Patent Application Laied-Open (Kokai) No. 153226/87) and so on have already been proposed as methods to increase the stability of IFN-β, and certain stabilizing effects have been achieved.

On the other hand, as with the IFN-β preparation, ointments which contain polyethylene glycol, propylene glycol, human serum albumin, polyvinyl pyrrolidone and methyl- or propyl-p-hydroxybenzoate as the base, are marketed by Serono (Italy) and Inter-Yeda (Israel), and are stated to be stable for 1 year when preserved at the low temperature of 4 to 8°C.

Although IFN-β can be stabilized by the above mentioned methods of the prior art, various additives such as viscosity-increasing agents or preservatives still need to be added to produce an ointment preparation of IFN-β. For example, when carboxymethyl cellulose "Daicel 2200" is added as the viscosity-increasing agent, IFN-β is remarkably less stable than when it is in 2 wt % of carboxymethyl cellulose ("Daicel 1240") - 50 wt % of glycerin - 48 wt % of 0.1 M citric acid buffer (pH 5) even if 4 kinds of IFN-β stabilizer such as 30 wt % or more (50 wt %, in this experiment) of glycerin, human serum albumin (International Patent Provisional Publication No. W083/01198), organic acid buffer (Japanese Patent Provisional Publication No. 92621/83) and 2 wt % of carboxymethyl cellulose ("Daicel 1240") (Japanese Patent Application Laied-Open (Kokai) No. 153226/77) are added. Furthermore, when p-hydroxybenzoates known as preservative are added, IFN-β will be inactivated as described in Japanese Application Laied-Open (Kokai)

Publication No. 176216/84.

The above mentioned preservation period of IFN-β, which is marketed in Italy and Israel, is 1 year when it is stored at 4 to 8 °C. However, it is necessary to develop a composition of IFN-β which is stable when stored at room temperature considering that the composition of IFN-β is to be sold in pharmacies and considering that it must be transported. Therefore, the purpose of this invention is to provide a prescription for a composition of IFN-β which is stable even at room temperature.

DISCLOSURE OF INVENTION

This invention is a stable composition of interferon-β containing 65 to 90 wt % of polyol and p-hydroxybenzoates.

A composition of IFN-β which can be stored for a long time at room temperature and in which IFN-β is not inactivated even if the composition contains various additives and in which the stabilization of IFN-β lasts for long time is obtained by this invention.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows the persistences of the titer of the IFN-β determined in EXAMPLE 1 to 4 and in COMPARATIVE EXAMPLE 1 to 4.

BEST MODE FOR CARRYING OUT THE INVENTION

In order to obtain a more stable composition of the IFN-β of this invention when the preservatives or the viscosity-increasing agents are added, the composition should contain 65 to 90 wt % of polyol, preferably polyol at 70 to 85 wt %.

The polyol in this invention is composed either of the alcohols of dihydric or of more hydric which contains propylene glycol, ethylene glycol, glycerin, polyglycerin or the like. These polyols can be employed either singly or in combination with two or more types.

The trihydric alcohols are preferred, and glycerin is especially recommended for the polyol in this invention.

As for the p-hydroxybenzoates used as the preservatives in this invention, methyl-, ethyl-, propyl-, butylester and so on can be cited. These p-hydroxybenzoates can be employed singly or in combination with two or more types. Ethyl p-hydroxybenzoate and propyl p-hydroxybenzoate are particularly favorable among the above cited p-hydroxybenzoates. The content of p-hydroxybenzoates is usually in the range of 0.01 to 0.2 wt %.

It is favorable that not only polyol and p-hydroxybenzoates, but also viscosity-increasing agents such as carboxymethyl cellulose and its salts, methyl cellulose, hydroxyethyl cellulose, starch, microfibrous cellulose and so on, and stabilizers such as human serum albumin, human serum

globulin and so on, be added when preparing the ointment. As for the viscosity-increasing agents, carboxymethyl cellulose or its salts are favorable, and those in which the viscosity of 1 wt % aqueous solution is 30 - 200 cps when measured by a B-type viscometer under conditions of 25 °C and 60 rpm are especially favorable. Specifically, "Daicel 1240", "Daicel 1260", "Daicel 1340", "Daicel 2200" (Daicel Chemical Industries, Ltd.) and so on of the sodium salt of carboxymethyl cellulose are recommended. The content of the viscosity-increasing agents used is usually in the range of 0.1 to 2.5 wt %. As for the stabilizer, human serum albumin, human serum globulin or the like is recommended, but human serum albumin is favored. The content of the stabilizer used is usually in the range of 0.1 to 1 wt %.

Furthermore, the mixing agents, such as the citric acid buffer and so on, can be mixed suitably.

The IFN-β mixed in the composition of this invention can be a compound produced by a cell culture or DNA recombinant technology if it is of human origin. The mixing amount of IFN-β is not specified, but it is favorable to mix IFN-β which has a titer of 1×10^4 IU/g or more.

This invention is further explained showing the embodiments. The measurement of the titer of interferon in the embodiments was performed by the method of cytopathic effect using Sindbis virus, VSV virus and the stabilized cell

line of human amnion origin (FL cells) and the obtained values were converted into international units (IU).

EXAMPLE 1

The IFN-β ["Feron", Toray] of the human diploid fibroblast origin produced by the cell proliferation treatment was used, and the composition of IFN-β is prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	70.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
Sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
Human serum albumin	0.60 %
0.1 M Citric acid buffer (pH 5)	26.88 %
IFN-β	1×10^5 IU/g

The sample of the composition of IFN-β prepared as above described is allowed to remain at 30 °C and the sampling was performed 4, 12 and 24 weeks after preparation. Its titer was determined and the persistence of the titer of the IFN-β was calculated based upon the initial titer of 100%. The obtained results are shown in Fig. 1.

EXAMPLE 2

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	75.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	21.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

EXAMPLE 3

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	80.00 %

Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	16.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

EXAMPLE 4

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	85.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
human serum albumin	0.60 %

0.1 M citric acid buffer (pH 5)	11.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

COMPARATIVE EXAMPLE 1

The same IFN-β as in Example 1 was used. The composition of IFN-β is prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	10.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	86.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

COMPARATIVE EXAMPLE 2

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	50.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose "Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	46.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

COMPARATIVE EXAMPLE 3

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	60.00 %

Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	36.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

COMPARATIVE EXAMPLE 4

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients, but some particle substances remained and this composition did not become homogenate. The composition ratios are represented as wt %.

Glycerin	95.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose	
"Daicel 1240"	2.00 %
"Daicel 2200"	0.50 %

human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	1.88 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Fig. 1.

EXAMPLE 5

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	80.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose "Daicel 1260"	2.00 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	17.38 %
IFN-β	1×10^5 IU/g

The sample of the composition of IFN-β prepared as above described was allowed to remain at 30 °C and the sampling was performed 12, 24, 36, 52 and 60 weeks after preparation. Its

titer was determined and the persistence of the titer of the IFN-β was calculated as the initial titer is 100%. The results obtained are shown in Table 1.

EXAMPLE 6

The same IFN-β as in Example 1 was used. The composition of IFN-β was prepared so that the composition contains the following ingredients. The composition ratios are represented as wt %.

Glycerin	80.00 %
Ethyl p-hydroxybenzoate	0.01 %
Propyl p-hydroxybenzoate	0.01 %
sodium carboxymethyl cellulose "Daicel 1340"	2.00 %
human serum albumin	0.60 %
0.1 M citric acid buffer (pH 5)	17.38 %
IFN-β	1×10^5 IU/g

This composition of IFN-β was stored the same as example 1 and the persistence of the titer of the IFN-β was calculated. The results obtained are shown in Table 1.

Table 1. Stability of EXAMPLE 5 and 6 at 30°C

Sample designation	Remaining titer (%)					
	initial	12 w	24 w	36 w	52 w	60 w
Example 5	100	94	100	104	83	80
Example 6	100	97	96	106	90	87

INDUSTRIAL APPLICAPABILITY

The composition of this invention is a composition of IFN-β which is stable for a long time at room temperature. Furthermore, the composition of this invention is an excellent composition in which IFN-β is not inactivated even if the composition contains various additives and the stabilization of IFN-β lasts for long time.

The composition of this invention can be prepared in various kinds of formulations, such as a liquid formulation, gel, spray, ointment and so on, but this composition is particularly suitable for ointments.

WHAT IS CLAIMED IS :

1. A stable composition of interferon-β containing 65 to 90 wt % of polyol and p-hydroxybenzoates.

2. A stable composition of interferon-β according to claim 1 wherein the content of p-hydroxybenzoates is 0.01 to 0.2 wt %.

3. A stable composition of interferon-β according to claim 1 wherein the p-hydroxybenzoate is ethyl p-hydroxybenzoate or propyl p-hydroxybenzoate.

4. A stable composition of interferon-β according to claim 1 wherein the content of polyol is 70 to 85 wt %.

5. A stable composition of interferon-β according to claim 1 wherein the polyol is comprised of the alcohols of dihydric or more hydric.

6. A stable composition of interferon-β according to claim 5 wherein the polyol is trihydric alcohol.

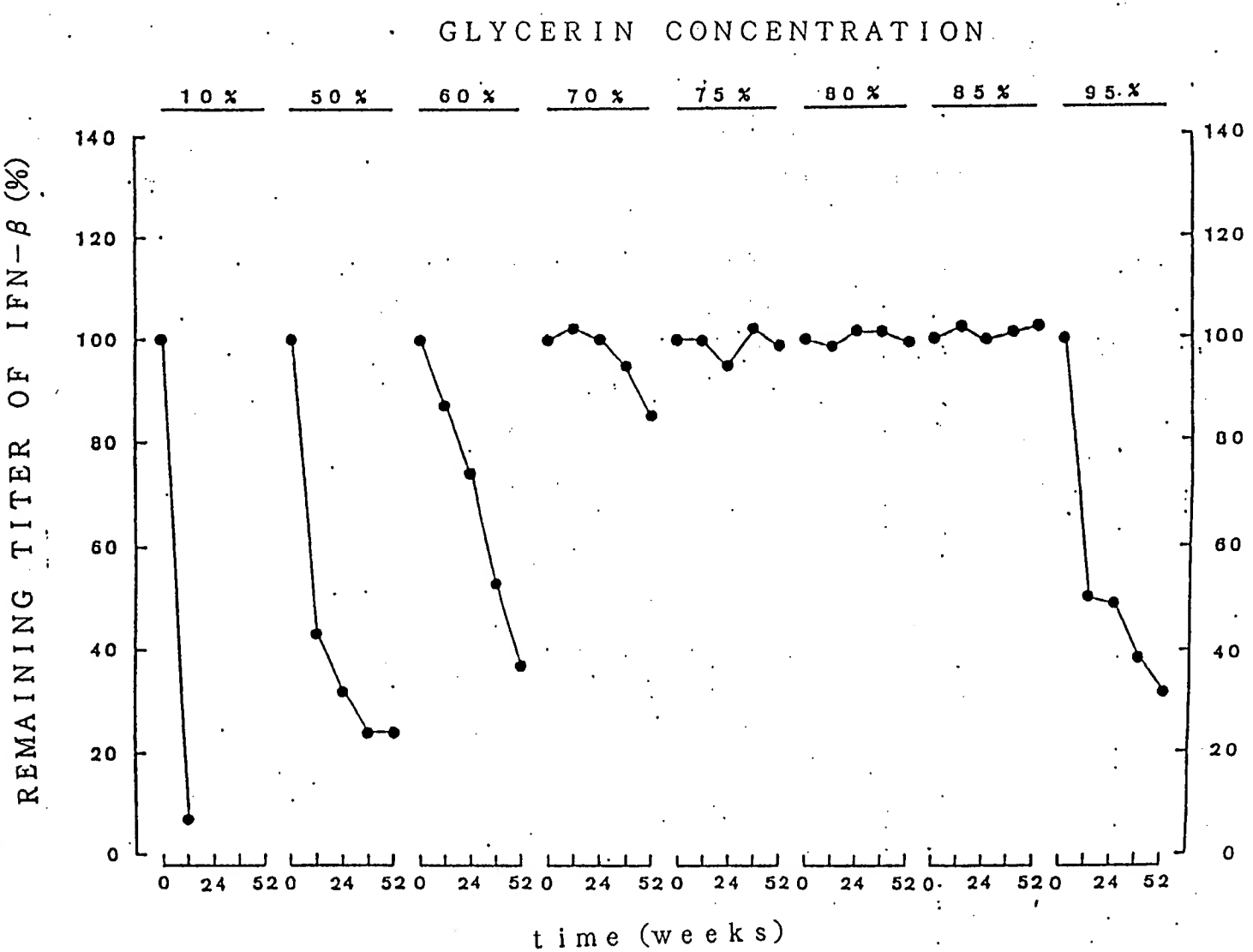
7. A stable composition of interferon-β according to claim 6 wherein the polyol is glycerin.

8. A stable composition of interferon-β according to claim 1 wherein the viscosity-increasing agent and the stabilizer are further contained.

9. A stable composition of interferon-β according to claim 8 wherein the content of the viscosity-increasing agent is 0.1 to 2.5 wt %.

10. A stable composition of interferon-β according to claim

FIG 1


GLYCERIN CONCENTRATION
10 %
50 %
60 %
70 %
75 %
80 %
85 %
95 %
REMAINING TITER OF IFN-β (%)
140
120
100
80
60
40
20
0
0 24 52
time (weeks)

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP89/00466

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[4]
A61K45/02, A61K47/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61K45/00 - 45/02, A61K47/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 62-209024 (K. Thomae GMBH) 14 September 1987 (14. 09. 87) Page 5, lower right column, lines 9 to 15 & EP, A1, 231816	1 - 17
A	JP, A, 60-69036 (Schering CORP) 19 April 1985 (19. 04. 85) Page 6, upper right column, lines 8 to 11 & EP, A1, 127130 & US, A, 4469228	1 - 17
A	JP, A, 62-153226 (Toray Industries, Inc.) 8 July 1987 (08. 07. 87) Claim (Family : none)	1 - 17
A	JP, A, 59-196823 (Sunstar Inc.) 8 November 1984 (08. 11. 84) Claim (Family : none)	1 - 17
A	JP, A, 58-92621 (Sunstar Inc. and Toray Industries, Inc.) 2 June 1983 (02. 06. 83) Claim & EP, A1, 80879 & US, A, 4675184	1 - 17

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
July 24, 1989 (24. 07. 89)	August 14, 1989 (14. 08. 89)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	